Applied Biology and Bioengineering (BT520)

EXPERIMENT- 4

PREPARATION OF COMPETENT CELLS

Aim: Preparation of fresh competent cells of *E. coli.*

Principle: The ability of the taking the DNA by a bacterial cell is called competence. *E. coli* cells can be made competent chemically. These cells are able to taken foreign DNA (recombinant plasmids or amplicons). The DNA is added to competent cells on ice. During a heat shock at 42°C the cells are transformed. Once the *E. coli* cells are transformed, the DNA can be extracted easily.

Requirements:

1. *DH5*α Host cells stock
2. Luria Bertani medium:
3. $MgCl_2$, 0.1 M (autoclaved)
4. $CaCl_2$, 0.1 M (autoclaved)
5. Ampicillin (100 mg/ml) filter sterilized

Luria Bertani medium: All the Ingredients are in (g/L)

Bactotryptone : 10.0g
Yeast extract : 5.0g
Sodium Chloride : 10.0g

adjust pH: 7.2 by NaOH or HCl

Equipment

1. Autoclave
2. Laminar Hood
3. BOD incubator shaker
4. Spectrophotometer
5. Ice Flaker
6. - 20/ -80°C deep freezer
7. Refrigerator
8.
9. Refrigerated centrifuge

Wares

1. Ice Bucket
2. Micropipettes
3. Cuvettes
4. Sterile centrifuge tubes (50 ml)
5. Sterile Eppendorf tubes (1.5 ml)
6. Autoclaved micro tips
7. Sterile Petri plates
8. Conical flasks 150/250 ml

Day 1:
Preparation of Media/solutions

1. Prepare LB-Agar medium for Petri plates (100 ml for 5 plates each group). (add 100 µg/ml ampicillin final concentration (100 µl from 100 mg/ml stock to 100 ml to cooled media to 40-45°C and pour 20 ml to each plate). Let the Petri plates cool down to room temperature and store them at 4-8°C in a refrigerator.
2. Prepare LB-liquid media (50 ml per group) in a 150/250ml conical flask
3. Prepare 0.1 M $MgCl_2$ (500 ml) and autoclave.
4. Prepare 0.1 M $CaCl_2$ (500 ml) and autoclave.

Inoculum preparation

Inoculate 100 µl of cells from frozen glycerol stock into 5 ml of LB medium. **OR** pick up one colony from petri plate using sterile toothpick). Grow the cells overnight at 37°C at 200 rpm.

Day 2:

Inoculation and Growth

1. Inoculate 1 ml of inoculum (from overnight culture) to 50 ml LB in conical flask and allow the cells to grow at 37℃, 200 rpm.

Cell harvest

2. Allow the cells to grow till $OD_{600\ nm}$ ~ 0.4-0.6 for about 2 hours.
3. Transfer the flask to ice and cool the cells for 10 min.

Competent cells

4. Centrifuge 40 ml culture from the flask in a 50 ml centrifuge tubes at 4000 rpm, at 4℃ 10 min. Discard the supernatant.
5. Resuspend the cell pellet gently first in 1-2 ml and then in 20 ml (in each tube) of 0.1 M $MgCl_2$ (Ice cold).
6. Centrifuge at 4000 rpm, at 4℃ 10 min. Discard the supernatant.
7. Resuspend cells gently in 2.0 ml (each tube) of 0.1 M $CaCl_2$ (Ice cold).
8. Leave the cells at 0℃ (on ice) for 2h.
9. The cells can be stored at 0- 4℃ for a week or use directly for transformation.

Observation:

Result: